

Tissue engineering in Oral & Maxillofacial surgery

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ABSTRACT

A recent innovation in dentistry is the preparation and use of platelet rich plasma, a concentration of platelets and growth factors found in platelets. These polypeptide growth factors as well as other bioactive substances are released from platelets upon activation, which play a pivotal role in initiating and sustaining wound healing and tissue repair mechanism. In order to improve wound healing and bone regeneration, attempts were made to increase the concentration of wound healing initiating factors in the form of platelet concentrate. Platelet derived polypeptide growth factors as well as other bioactive substances are released from platelet upon activation which plays a pivotal role in initiating and sustaining wound healing and tissue repair mechanism. This article is an attempt to highlight the uses of Platelet rich plasma (PRP) in Oral & Maxillofacial Surgery and the preparation of PRP gel in the immediate preoperative period in a laboratory centrifuge.

Key Words: Tissue engineering, platelet gel, bone grafting, maxillofacial surgery

Introduction

Within the past decade, a new field of "tissue engineering" or "regenerative medicine" has emerged that offers a new and exciting alternative for maxillofacial surgery and reconstruction. It offers our specialty a new option to supplement existing treatment regimens for

reconstruction/ regeneration of the oral & craniofacial complex, which includes the teeth, periodontium, bones, soft tissues (oral mucosa, conjunctiva, and skin), salivary glands and the temporomandibular joint, as well as blood vessels, tendon, muscles and nerves.

Tissue engineering / regenerative medicine is a novel field that draws support from multiple disciplines,

including molecular, cellular biology and physiology.

Platelet Rich Plasma (PRP) - An Insight

PRP was first introduced to oral surgery community by Whitman et al^[1] in their 1997 article entitled "Platelet Gel: An autologous alternative to fibrin glue with application in Oral & Maxillofacial Surgery." The authors thought that through activation of the platelets in within the gel and the resultant release of growth factors, enhanced wound healing should be expected". PRP enjoyed a great deal of popularity in Oral & Maxillofacial Surgery after the publication of a landmark article by Marx et al^[2] in 1998. Marx et al study showed that combining PRP with autogenous bone in mandibular continuity defects resulted in significantly faster radiographic maturation and histomorphometrically denser bone regenerate. It certainly seemed as though a new age in bone grafting had begun.

Platelet rich plasma can be procured in the immediate preoperative period by various techniques. In the literature, techniques of PRP preparation vary from using 10 cc of a patient blood and spinning it in a lab centrifuge,^[3] to utilizing a unit of blood (350-500) that is put through a cell separator that sequester and concentrate the platelets.^[2, 4]

Platelet rich plasma [PRP] offers upto a 2.16 times increase in the maturation rate and substantially greater density of a bone graft regenerate.^[2] Soft tissue healing is also substantially improved through the application of PRP, by increasing collagen content, promoting angiogenesis and by early wound strength^[3, 5, 6]

PRP has been shown to contain various growth factors, including platelet derived growth factor, transforming growth factor- β (TGF- β), vascular

endothelial growth factor (VEGF), platelet derived endothelial growth factor (PDEGF) and fibroblast growth factor (FGF). These polypeptide growth factors as well as other bioactive substances are released from platelets upon activation and play a pivotal role in initiating and sustaining wound healing and tissue repair mechanism.^[2, 5, 7, 8]

The activation of PRP mimics the final stages in the clotting cascade and results in a gelatinous substance known as PRP gel. This activation of PRP can be done by various means to form PRP gel or can be used on its own. The PRP can be activated by calcium alone, or autologous whole blood and some autogenous cancellous bone, both containing thrombin, or a mixture of 10% CaCl₂ and bovine thrombin.^[9, 10] However, few studies have reported hypersensitivity reaction with the use of bovine thrombin.^[11]

What is PRP and Platelet Gel?

Platelet Rich Plasma is an autologous concentration of human platelets in a small volume of Plasma. True PRP is always autologous and is not homologous. Homologous platelets are not viable and could not possibly secrete bioactive growth factors. Homologous platelets are also antigenic due to their abundance of cell membranes. Certainly, antiplatelet antibodies could develop from this product and second set reactions would follow.

How does PRP work?

Platelets are our primary mechanism for hemostasis. They circulate in our bodies looking for exposed endothelium. They then aggregate to the site of injury and further platelet degranulation occurs. The release various growth factors can also aid in the healing process. Platelet gel mimics the final stages in the clotting

cascade. The platelet rich plasma in the presence of thrombin activates platelets, converts fibrinogen to fibrin and stimulates further platelet aggregation. Calcium chloride is added to counteract the anticoagulant citrate, rapidly forming gelatinous, platelet-rich glue platelet aggregation.

PRP works via the degranulation of the alpha granules in platelets which contain the synthesized and pre packaged growth factors. The active secretion of these growth factors is initiated by the clotting process of blood and begins within 10 minutes after clotting. Therefore PRP must be developed in an anticoagulated state and should be used on the graft, flap, or wound within 10 minutes of clot initiation.

Like most growth factors such as bone morphogenic protein, the growth factors within the alpha granules of platelets are incomplete because they must be soluble. As the clotting process activates the platelets, the growth factors are secreted from the cell through cell membrane. (In this process, the alpha granules fuse to the platelet cell membrane where the protein growth factor is completed to a bioactive state by the addition of histones and carbohydrate side chain to these proteins.)

The secreted growth factors immediately bind to the external surface of the cell membrane of various cells in the graft, flap or wound via trans - membrane receptors. Studies have shown that adult mesenchymal stem cells, osteoblasts, fibroblasts, endothelial cells and epidermal cell express cell membrane receptors to growth factors in PRP. These transmembrane receptors in turn induce an activation of an endogenous internal signal protein, which causes the expression of a normal gene sequence of the cell such as cellular proliferation, matrix formation, osteoid production,

collagen synthesis etc. After the initial burst of PRP related growth factors, the platelets synthesize and secrete additional growth factors for the remaining 7 days of their life span.

Once the platelets are exhausted and die off, the macrophages arrive at the region and assume the function of wound healing regulation by secreting some of the growth factors as well as others. Therefore the number of platelets in the blood clot within the graft, wound, or adherent to a flap sets the rate of wound healing.

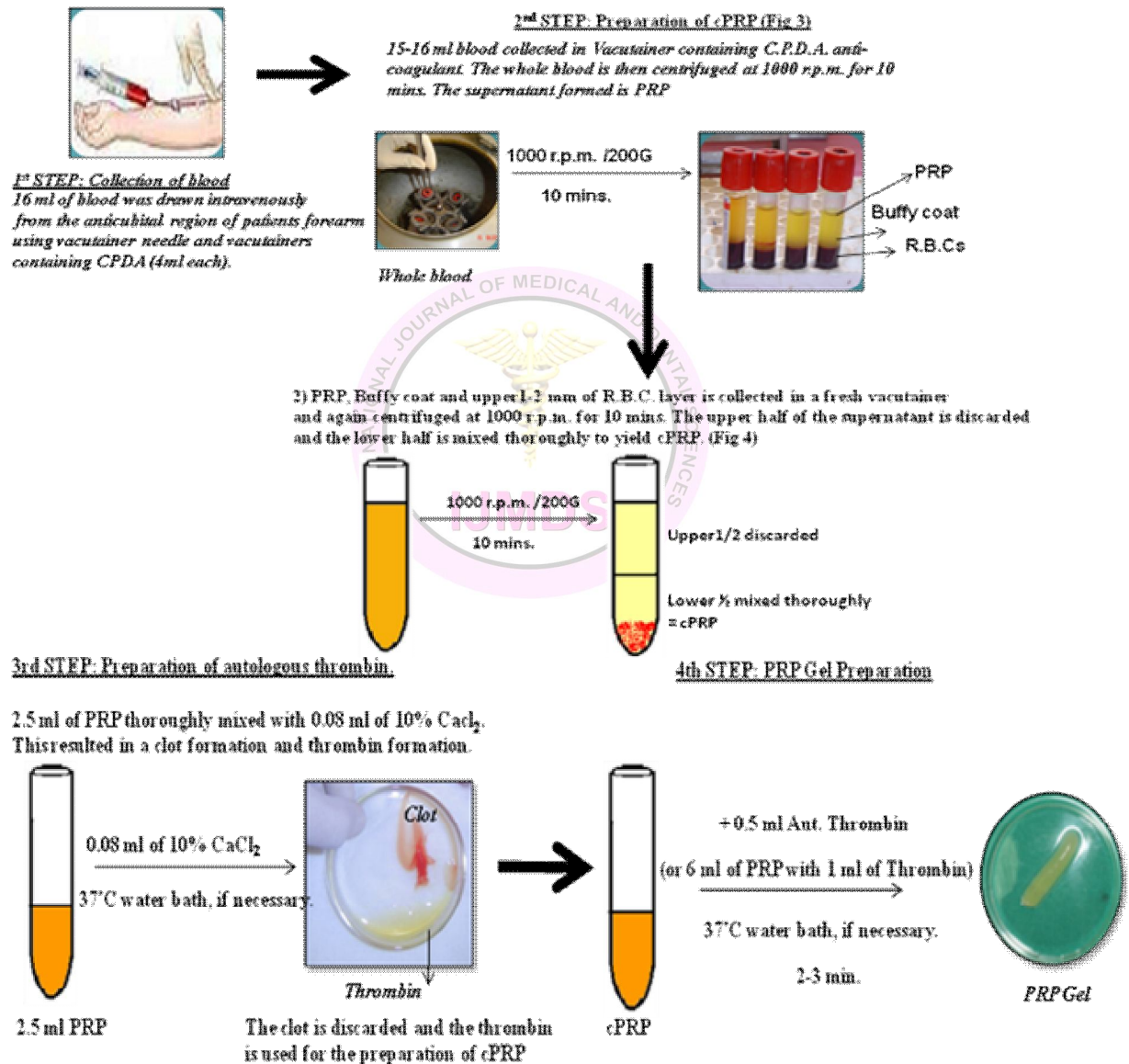
Preparation of PRP Gel: There are various methods for preparing PRP. Techniques vary from using 10c.c of patient's blood and spinning it in a clinical centrifuge,^[3] to using a unit (400-500 c.c) of blood that is put through a cell separator that sequesters and concentrates the platelets.^[2, 4] In our Institute, PRP was prepared from 16 cc of patient's blood and centrifuging the blood twice in a clinical centrifuge^[12] and also advocated by Regina Landesberg.^[13]

The PRP is activated to form PRP gel thus causing degranulation of α -granules present in the platelets and releasing the growth factors. The various agents for the activation reported in literature include CaCl_2 alone, CaCl_2 plus bovine thrombin, Human Thrombin, autologous bone or whole blood which contains thrombin.^[9] Regina Landesberg^[11] has reported the possibility of hypersensitivity to bovine thrombin which may cause antigenic reactions in many individuals. In our technique autologous thrombin was prepared and was mixed with PRP to form an autologous platelet gel.^[12] This platelet gel was free of eliciting any antigen-antibody reaction as it was prepared from patients own blood.

The positive effects of PRP as reported in literature are: [2, 5, 7, 8]

- “Jump-starts” the cascade of osteogenesis in a bone graft.
- Promotes early consolidation of the graft
- Speeds up mineralization of the graft.
- Improves trabecular bone density.
- Allows placement of implants into the graft at an earlier time.
- Provides earlier availability of growth factors and BMP.
- Enhances osteoconduction

PREPARATION OF PRP GEL [12]



Applications of PRP in oral & Maxillofacial Surgery

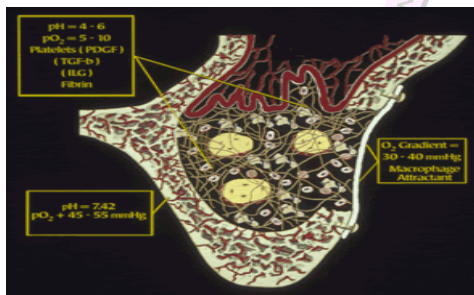
Split Thickness Skin Graft Donor Sites: [7, 8]

PRP has demonstrated efficacy in the healing of split thickness skin graft (STSG) donor sites. The revascularization is quickly enhanced by the angiogenic activity of PDGF and TGFβ.

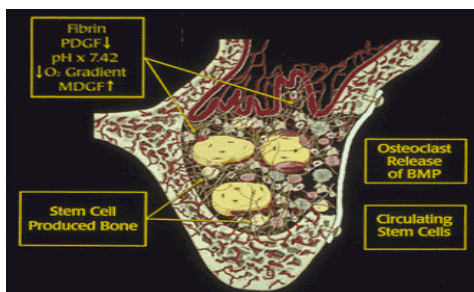


Donor site showing healing of split thickness skin graft

Sinus Lift Grafts: [15] PRP Gel also improves the handling of particulate graft apart from enhancing the osteogenesis of graft



Physiology of sinus lift graft with platelets enmeshed in graft



Bone formation and healing of autogenous sinus lift

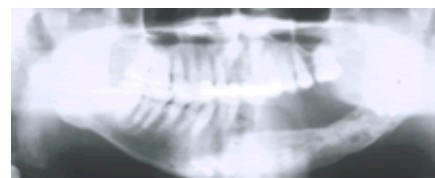
Ridge Augmentation Graft: [14, 15] Both vertical and horizontal ridge

augmentation procedures will benefit from PRP. If either a cortical-cancellous block or a strictly cancellous marrow graft is used the PRP is incorporated into and on the surface of the graft.

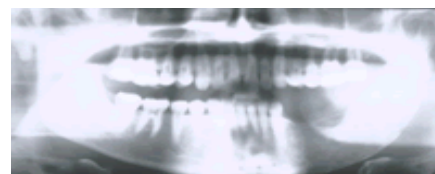
Continuity Defects in the Jaws: [5, 7]

In the context of continuity defects these grafts are accomplished in an operating room setting. The PRP should be developed prior to the infusion of large fluid volumes which will dilute blood components and prior to any significant tissue wounding which will sequester platelets in the wound. The PRP may remain on the sterile field in an anticoagulated state for up to 8 hours. However, once "activated" with calcium and a clot initiator, it should be directly used.

The nature of large continuity grafts recommends incorporating the PRP into the graft during placement with a layering technique. That is, small amounts of PRP gel are added to the graft as it is placed. It is then best to place some on the graft surface. About 20 cc's to 35 cc's of PRP are usually required depending on the size of the graft.



Normal bone density radiographically observed in graft without PRP at 4 months



Increased bone density radiographically observed with PRP enhanced graft at 4 months

The activated PRP gel may be made into a bio-resorbable membrane which will last for approximately five to seven days. This is accomplished by "activating" the PRP into a gel and placing 3 ml to 4 ml on a smooth surface. After approximately five minutes the PRP gel can be taken off the surface as a membrane. This membrane will consist of fibrin, in which is enmeshed the platelets. It may be used over sinus lift windows, to cover sinus membrane perforations, or over dental implant fixtures.

Periodontal surgery ^[14, 17]



Before grafting



Grafted Freeze dried cortical bone allograft

A study was conducted in which the freeze dried cortical bone allograft was grafted into wide three wall, two wall, and one wall combination furcation defects. The authors concluded that out of 97 defects treated, 23 manifested complete bone regeneration, 30 showed greater than 50%, 24 less than 50% osseous repair and 12 defects failed to demonstrate any regeneration, of which nine were furcation involvement.

PRP Added to Commercial Membranes:

Commercial Membranes such as Collatape®, Resolute®, or Ossequest®, have a texture which will absorb the "activated" PRP gel. This will allow the clinician to apply growth factors to longer lasting membranes so as to gain the benefit of each.

Extraction sockets ^[3, 8, 20]



Platelet rich plasma placed in the extraction sockets after third molar surgery

A study on 117 patients was done in which platelet rich plasma was placed in the extraction sockets after third molar surgery. The result showed a decrease rate of alveolar osteitis in sockets treated with PRP. The PRP treated sockets also showed better hemostasis, faster soft tissue flap healing, and decreased swelling post operatively. One month postoperative radiographs showed subjectively more dense bone fill and radioopacity in the PRP treated sockets. ^[20]

Alveolar Bone Grafting ^[16]



Figure shows Alveolar bone grafting

In a study on 7 Cleft lip and palate patients, the cleft alveolus was grafted with autologous iliac cancellous bone incorporated with platelet rich plasma (PRP). The bone regenerate at the cleft site was quantitatively evaluated using 3 dimensional computed tomography scans at 5 or 6 months postoperatively. The results showed a higher volume ratio of regenerated bone to alveolar cleft in cases treated with PRP than in controls. ^[16]

PRP in Implant surgeries

In a case presented by Thor A ^[19] in the year 2002, particulated autogenous bone, platelet gel, and a titanium mesh were used for alveolar bone reconstruction of the anterior maxilla prior to implant placement. After 4.5 months of healing the mesh was removed and titanium implants were placed. The results showed that the healing was uneventful, and the anterior maxilla had increased in height and width during the initial healing. All implants became integrated and supported a fixed dental bridge for over 3 years with no dramatic dimensional changes of the graft. It was concluded that the autogenous growth factors in the gel possibly contributed to the positive outcome.

Distraction osteogenesis

Robiony M, Polini F, Costaf, Poloti M ^[18] evaluated a new method on restoring severe atrophic mandible using platelet-rich plasma (PRP) during distraction osteogenesis. During the surgery, a mixture of autologous iliac bone graft and an autologous platelet concentrate filled the distraction gap. This mixture constituted an autologous bone-platelet gel that was used to create a useful bony scaffold for distraction regenerate. After a latency period of 15 days, a distraction run of 0.5 mm/d, and a 60-day period of consolidation, the distraction device was

removed and implants were placed simultaneously. The results showed that in all the treated patients, planned distraction height was achieved with a considerable enhancement of bony regeneration, and in all cases it was possible to place implants at a planned time. The study concluded that the combination of these recent and innovative regenerative methods seems to be effective in restoring the severe atrophic mandible.

PRP in patients with anti coagulants.

Antonio Della Valle et al. ^[21] in their study had put PRP gel in the extraction socket in 40 patients on anti coagulant drugs (suspended 36 hrs prior to the extraction). The results showed that only 2 patients reported hemorrhagic complications (5%). Sixteen patients (40%) had mild bleeding that was easy to control with hemostatic topical agents.

The remaining 22 patients (55%) presented with adequate hemostasis. Thus it was concluded that, oral surgery in cardiac patients under oral anticoagulant therapy might be facilitated with PRP gel. This biological and therapeutical improvement can simplify systemic management and help avoid hemorrhagic and/or thromboembolic complications.

Conclusion

In surgery, tissue engineering is a relatively new and promising field, which can promote wound healing by incorporating the recent advances in molecular, cellular biology and physiology. According to literature platelets are rich in growth factors that may contribute to an accelerated tissue regeneration process. In order to improve wound healing and bone regeneration, attempts were made to increase the concentration of wound healing initiating factors in the form of platelet concentrate.

Platelet derived polypeptide growth factors as well as other bioactive substances are released from platelet upon activation which plays a pivotal role in initiating and sustaining wound healing and tissue repair mechanism.

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